Dissolved lignin within the waters of Barkley Sound, Vancouver Island, Canada

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Non-technical Summary:

Terrestrial plants produce lignin in their tissues which consists of a variety of smaller units such as syringyl, cinnamyl and vanillyl lignin phenols. The concentration and type of the lignin phenols depends on the tissue type and species of vascular plant. So a plant signature of the lignin is obtained by comparing the individual lignin phenol ratios. The plant signature represents the plant origin of the lignin which can be traced back to evergreen/deciduous wood, needles, leafs or grasses. This study uses dissolved lignin phase as a biomarker to determine the plant sources of terrestrial derived organic carbon that enters into the waters of Barkley Sound, Vancouver Island. The ratio between the individual lignin phenols indicates that 3/4 of the lignin originated from woody tissue rather than needle and 3/4 of the woody lignin came from an evergreen wood rather than a deciduous wood. However, lignin in the dissolved phase undergoes much biological degradation with in the soil prior to arriving in Barkley Sound. This degradation alters the lignin phenol ratios that we obtained in the estuarine waters and alters the plant signature given by the lignin phenols. Considering the alteration, a generous signature of lignin phenols at most 4/5 the lignin could come from needle instead of wood, while only 1/2 could originate from deciduous wood instead of evergreen wood.

Acknowledgements

I would like to thanks lot of people who helped me in the various processes of this thesis trip. For taking the tame of reading massive load of papers and giving feedback I would like to appreciate Deborah Kelly, Colleen Durkin, Rick Keil. In the pursuit of laboratory skills, especially learning to work a fancy microwave, I thank Jaqui Neibauer. For making this experience physically possible I would like to thanks the Bamfield Marine Center for ability to do field work and Rick Keil labs in providing all the necessary lab equipment. Lastly, I have to thank the whole senior class in making this whole trip fun.

Abstract:

This study uses dissolved lignin as a biomarker to determine the plant sources of terrestrial organic carbon in Barkley Sound, Vancouver Island. The lignin concentration follows a typical decreasing trend from head of the inlets to the ocean. The total concentration of the syringyl, cinnamyl and vanillyl lignin phenols is considerably small compared other part of the world. The ratio of syringyl to vanillyl phenols (S/V) and cinnamyl to vanillyl phenols (C/V) indicates that 3/4 of the lignin originated from woody tissue rather than needle and 3/4 of the woody lignin came from an evergreen rather than a deciduous wood. However, the ratio of syringic acid to Syringaldehyde (Ad/Al(s)) was higher then vanilllic acid to vanillin (Ad/Al(v)). These Ad/Al indicate the degradation of lignin which lower the S/V and C/V ratios. A lignin phenol vegetation index (LPVI) value of 75 was produced upper limits S/V and C/V ratios that indicate lignin signatures of plant material before being degraded. The upper limits show that at most 4/5 the lignin could come from needle instead of wood, while only 1/2 could originate from deciduous wood instead of evergreen wood.

Introduction:

To understand the global carbon cycle, it is important to also understand both the marine organic carbon and terrestrial organic carbon (OC) pathways and interactions in the environment. Terrestrial OC transported to coastal and estuarine areas by river systems (Walsh et al. 2008). Nuwer and Keil (2005) document the importance of estuaries as an important sink for the majority of terrestrial OC in the ocean. Additionally, the size difference of particulate and dissolved OC leads to separate times and pathways at which the terrestrial OC is transported to these estuaries. (Hernes et al. 2007). This leads to different degrees of both biological and physiochemical degradation. These interactions emphasize the importance of understanding the composition, sources and distribution of terrestrial OC in estuaries.

Since Terrestrial OC influences is a major component in the total OC inventory of estuarine systems, it largely influences local biological processes (Walsh et al. 2008). A study by Hopkinson et al. (1998) illustrates that the concentration of dissolved terrestrial OC increases the bioavailability of carbon and therefore increases bacterial growth. In contrast, there is a decrease in bioavailability with higher concentrations of particulate terrestrial OC. Additionally, different terrestrial plants produce organic molecules of varying stability. This is significant because the greater stability of some molecules, such as lignin, leads to lesser bioavailability rates (Hopkinson et al 1998). Understanding regional effects of terrestrial OC on bacterial growth rates would further our knowledge on nutrient recycling in the marine ecosystems.

Lignin phenols are also widely used as a biomarker for terrestrial OC because only terrestrial vascular plants produce lignin (Hedges and Mann 1979). The ratios of syringyl, cinnamyl and vanillyl phenols to one another can be used to indicate the source of organic matter. The ratios of different lignin phenols to one another vary depending on the species and of the type vascular plant tissue from which they originated. Lignin ratios most commonly divide terrestrial OC into woody/ non-woody angiosperm or gymnosperm plant categories (Hedges and Ertel 1982). Thus, analysis of lignin phenol ratios is a powerful tool to determine source of the total terrestrial OC.

Both particulate and dissolved lignin phases can used to analyze to source of terrestrial OC. But the biological, physical and chemical processes affect the signal source from the ananlysis. A study by Hernes et al (2007) showed that dissolved lignin is characteristic of being leached from the parent soil and varies with lignin phenols. This one physical property creates fractionation between particulate and dissolved lignin in freshwater input (Hernes et al. 2007). Lignin that is eventually dissolved in ground water spends more time in the soil being biologically degraded. Thus when freshwater enters marine waters the particulate and dissolved lignin may not be representative of the same geographical/ temporal origin, plant origin or degree degradation processes. Dissolved lignin could be a better indicator for understanding the degradation processes of terrestrial OC then particulate lignin.

No studies have been conducted on dissolved lignin in the water-column of temperate estuarine systems. The major lignin source is likely gymnosperm wood because the terrestrial landscape is dominated by evergreen forests. This study will focus on the lignin phenol signature obtained in Barkley Sound and how degradation affects this signature. The study can be further applied to the lager Pacific Northwest region, as many of the estuaries exist under similar conditions.

Methods:

Study Area:

Barkley Sound is fjord-like estuary located in the southwest part of Vancouver Island (Fig. 1). Study areas within Barkley Sound include Effingham Inlet, Alberni Inlet, San Mateo Bay, Sarita Bay, Trevor Channel and Imperial Eagle Channel. Effingham Inlet is a fjord separated from Imperial Eagle Channel by a 65 m sill at the inlet entrance. A 40 m sill further separates Effingham Inlet into an inner and outer basin with depths of 120 and 210 m, respectively (Hay et al. 2003). Fresh water into the inlet comes from Effingham River, Coeur d'Alene Creek and non-point sources from walls of the fjord (Hay et al. 2003). The sills cause weak mixing and strong stratification. This stratification allows anoxic waters to form in the inner basin and sub-anoxic in the outer basin. With the exception of Effingham Inlet, Barkley Sound exhibits a stronger mixing and circulation and is therefore less likely to become anoxic.

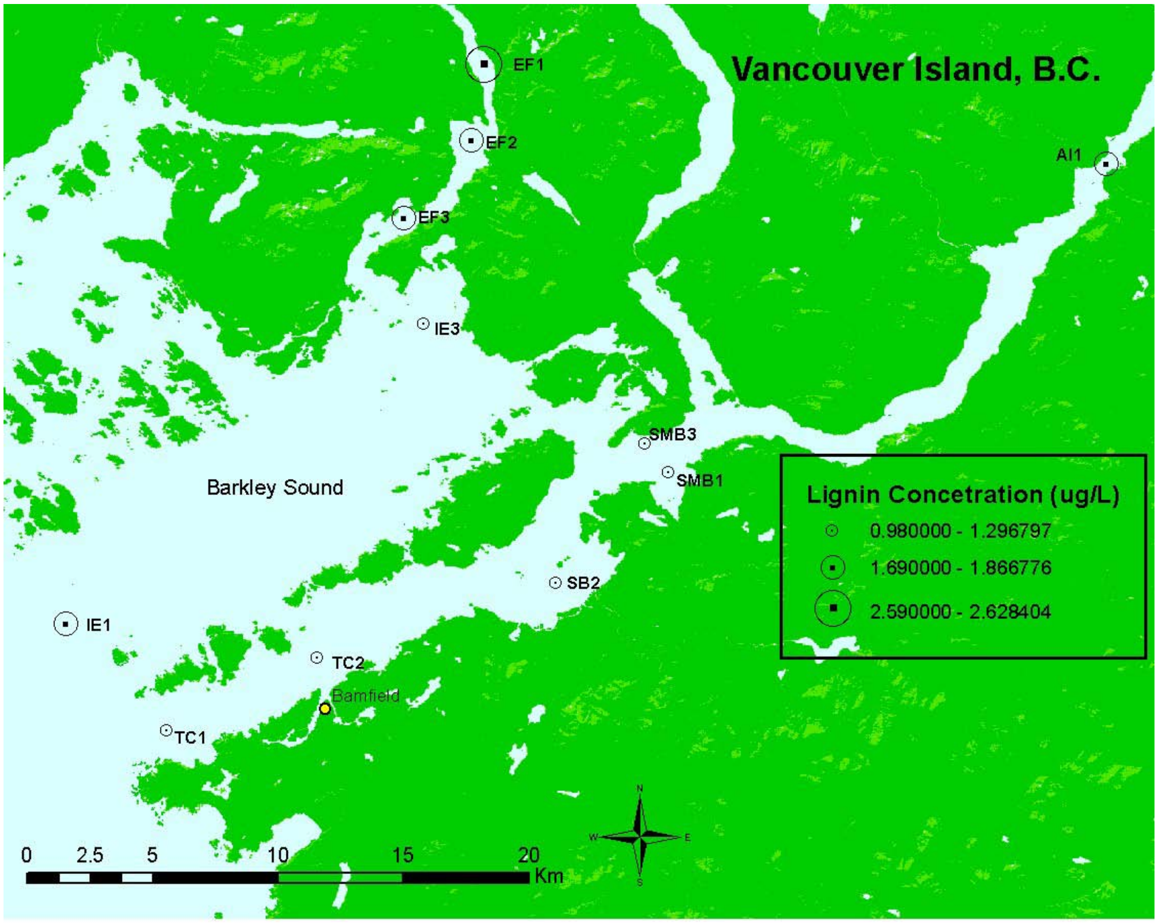


Figure 1
Map of Barkly Sound in Vancouver Island, B.C. and stations sampled. Circles represent the averaged total dissolved lignin concentration of all depths sampled at each location.

Field Work:

The research in Barkley Sound was conducted aboard the R/V Barkley Star, March 22-25, 2010, including a total of 12 stations (Fig. 1). Water samples of 4 liters were collected at all sampled depths. The 0 m samples in Effingham Inlet were taken directly in 4 liter plastic containers. Samples at 5 m depth were taken by hand niskin. Deep water samples were taken in niskin bottles on a handheld line with the aid of an electric motor system. A Seabird CTD was used on the same line to obtain depth.

Water samples were then filtered using a 47 mm GF/F filter to separate the particulate and dissolved terrestrial organic carbon. The 4 liters of each filtered water sample were acidified to pH=2 with HCl and an internal standard of 3, 4 dihydroxybenoic acid was added to a concentration of 2.6 mg L^1 (Keil and Neibauer 2009). For the solid phase extraction of lignins, approximately 4 liters of each sample was run through an Oasis Waters HLB cartridge pretreated with 1 ml of methanol, 1 ml of ethyl acetate and acidified 1M MilliQ water at 3 ml m^{-1}. Following sample loading, 2ml of acidified water were passed through the cartridges to keep the columns moist before being stored at 4°C.

Lab Work:

The cartridged samples were eluted with 5 ml of ethyl acetate at 1 drop s^{-1} to extract dissolved lignin (Keil and Neibauer 2009). Then 2.5 ml of the extract was put into a teflon vessel to dry under N_2 gas. The other half of the dissolved lignin extract was saved for duplicate analysis. Next, lignin samples underwent CuO oxidation with a microwave digestion apparatus to break apart individual phenols (Goni and Montgomery 2000). The lignin was extracted by the addition of HCL and ethyl acetate. Dried lignin samples were then re-suspended in pyridine. Finally, N,o-Bis (Trimethylsilyl) trifluoroacetamide and trimethylchlorosilane were added to samples before analyzed by a Gas Chromatography with a Flame Ionization Detector (GC-FID) (Goni and Montgomery 2000). Individual Lignin phenols were quantified and identified by comparing their retention times and peak areas to those of known standards.

Data Analysis:

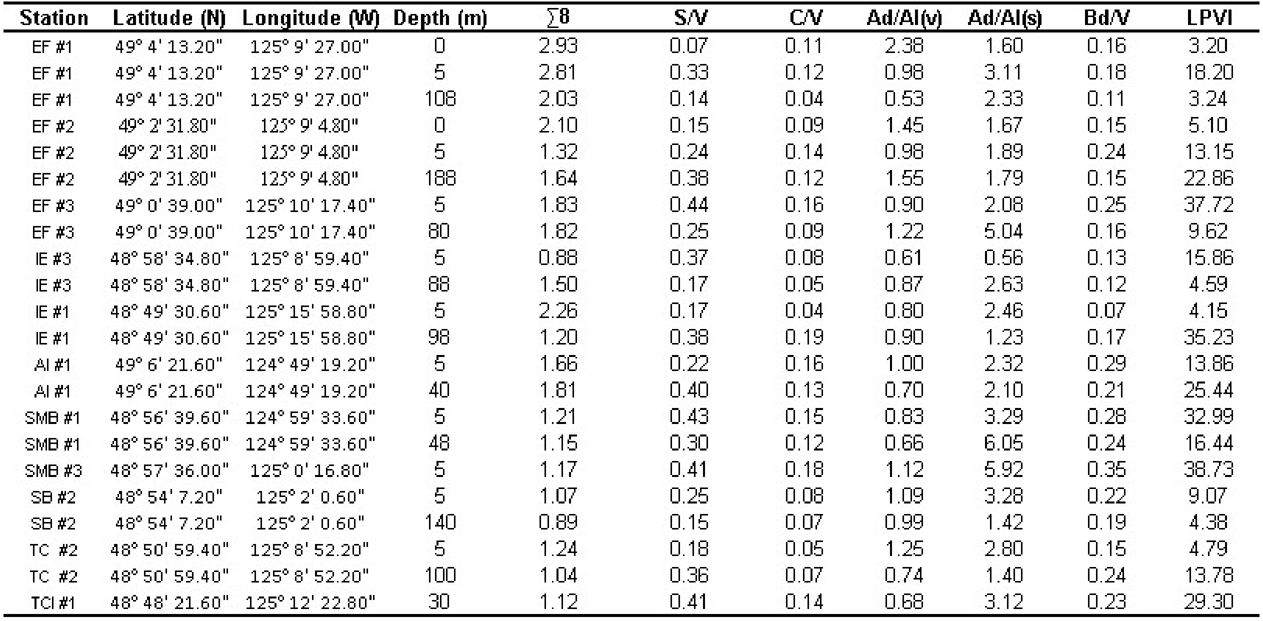
Analysis of the water samples quantified dissolved concentrations of syringyl (S), vanillyl (V) and cinnamyl (C) lignin phenols (Goni and Montgomery 2000). Hedges and

Mann (1979) describes that vanillyl phenols are produced by both woody and non-woody vascular tissue of angiosperm and gymnosperm (Fig. 2). An angiosperm is distinguished from a gymnosperm by a (S/V) ratio higher then 0 because only they produce syringyl phenols. Furthermore, gymnosperms and angiosperms can be classified into wood and non-woody vascular tissue. The non-woody tissues produce cinnamyl phenols and woody tissue does not. Therefore non-woody tissues can be identified with a non-zero (C/V) ratio. Plotting syringyl/vanillyl (S/V) to cinnamyl/vanillyl (C/V) lignin phenol ratios will be used to determine the source of terrestrial OC for a single sample.

Results:

The total dissolved lignin concentrations range from 0.88-2.93 µgL^{-1}. The highest concentrations, 2.81-2.93 µgL^{-1}, are seen in all depths sampled at station EF #1. The lowest concentrations of 0.88-1.20 µgL^{-1} are located at ocean influenced waters; Imperial Eagle Channel, Trevor Channel, San Mateo Bay and Sarita Bay. Station IE #1 at 5 meters exhibits a 2.26 µgL^{-1} concentration which is higher than other ocean stations. The station in Alberni Inlet, the furthest from ocean waters

Table 1
Dissolved lignin values of samples in Barkly Sound. Sum 8 (∑8) as µgL^{-1}; S/V, ratio of syringyl to vanillyl phenols; C/V, ratio of cimmonyl to vanillyl phenols; vanillic acid/ vanillin; syringic acid/ syrinaldhyde; Bd/V, ratio of 3,5 dihydroxybenzoic acid to vanillyl phenols; LPVI.



Station	Latitude (N)	Longitude (W)	Depth (m)	∑8	S/V	C/V	Ad/Al(v)	Ad/Al(s)	Bd/V	LPVI
EF #1	49° 4' 13.20"	125° 9' 27.00"	0	2.93	0.07	0.11	2.38	1.60	0.16	3.20
EF #1	49° 4' 13.20"	125° 9' 27.00"	5	2.81	0.33	0.12	0.98	3.11	0.18	18.20
EF #1	49° 4' 13.20"	125° 9' 27.00"	108	2.03	0.14	0.04	0.53	2.33	0.11	3.24
EF #2	49° 2' 31.80"	125° 9' 4.80"	0	2.10	0.15	0.09	1.45	1.67	0.15	5.10
EF #2	49° 2' 31.80"	125° 9' 4.80"	5	1.32	0.24	0.14	0.98	1.89	0.24	13.15
EF #2	49° 2' 31.80"	125° 9' 4.80"	188	1.64	0.38	0.12	1.55	1.79	0.15	22.86
EF #3	49° 0' 39.00"	125° 10' 17.40"	5	1.83	0.44	0.16	0.90	2.08	0.25	37.72
EF #3	49° 0' 39.00"	125° 10' 17.40"	80	1.82	0.25	0.09	1.22	5.04	0.16	9.62
IE #3	48° 58' 34.80"	125° 8' 59.40"	5	0.88	0.37	0.08	0.61	0.56	0.13	15.86
IE #3	48° 58' 34.80"	125° 8' 59.40"	88	1.50	0.17	0.05	0.87	2.63	0.12	4.59
IE #1	48° 49' 30.60"	125° 15' 58.80"	5	2.26	0.17	0.04	0.80	2.46	0.07	4.15
IE #1	48° 49' 30.60"	125° 15' 58.80"	98	1.20	0.38	0.19	0.90	1.23	0.17	35.23
AI #1	49° 6' 21.60"	124° 49' 19.20"	5	1.66	0.22	0.16	1.00	2.32	0.29	13.86
AI #1	49° 6' 21.60"	124° 49' 19.20"	40	1.81	0.40	0.13	0.70	2.10	0.21	25.44
SMB #1	48° 56' 39.60"	124° 59' 33.60"	5	1.21	0.43	0.15	0.83	3.29	0.28	32.99
SMB #1	48° 56' 39.60"	124° 59' 33.60"	48	1.15	0.30	0.12	0.66	6.05	0.24	16.44
SMB #3	48° 57' 36.00"	125° 0' 16.80"	5	1.17	0.41	0.18	1.12	5.92	0.35	38.73
SB #2	48° 54' 7.20"	125° 2' 0.60"	5	1.07	0.25	0.08	1.09	3.28	0.22	9.07
SB #2	48° 54' 7.20"	125° 2' 0.60"	140	0.89	0.15	0.07	0.99	1.42	0.19	4.38
TC #2	48° 50' 59.40"	125° 8' 52.20"	5	1.24	0.18	0.05	1.25	2.80	0.15	4.79
TC #2	48° 50' 59.40"	125° 8' 52.20"	100	1.04	0.36	0.07	0.74	1.40	0.24	13.78
TCI #1	48° 48' 21.60"	125° 12' 22.80"	30	1.12	0.41	0.14	0.68	3.12	0.23	29.30

The S/V values range from 0.07-.044 with a mean of 0.28 and C/V values range from 0.4-0.19 with a 0.11 mean (Table 1). An S/V to C/V plot produced all stations consisting of single cluster with S/V < 1 and C/V < 0.2 (Fig. 2).

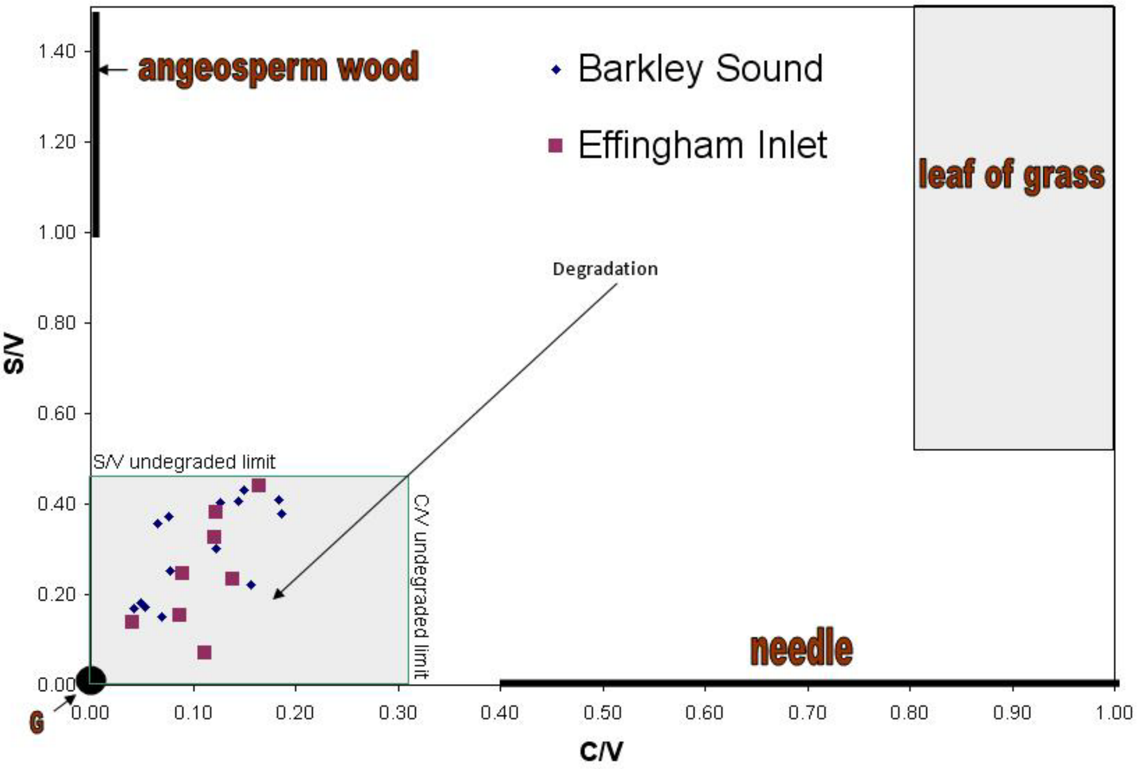


Figure 2

Plot of syringyl to vanillyl phenols (S/V) vs cinnamyl to vanillyl phenols (C/V) providing incite on lignin's terrestrial origins. Area represents of angiosperm wood (deciduous trees), (G) gymnosperm wood (evergreen wood).

Aldehyde to alcohol ratios of vanillyl (Ad/Al(v)) and syringyl (Ad/Al(s)), and Lignin Phenol Vegetation Index (LPVI) are given in table 1.

Discussion

The sequestration of terrestrial OC occurs mainly in estuarine systems. This happens because the highest dissolved lignin concentration originates from its land based freshwater sources. As dissolved lignin travels toward the ocean it adsorbs to mineral surfaces, which then settles out of the water column (Mayer 1994). Therefore, the smallest dissolved lignin concentrations appear in the ocean waters. This is apparent in Barkley Sound with a dissolved lignin concentration of 3.9 μgL^{-1} and 1.6 μgL^{-1} at the head of Effingham Inlet and oceans waters, respectively. In contrast, the inner most station AI# 1 has a dissolved lignin concentration of 2.4 μgL^{-1}. The AI#1 station is located in the center of a 14 km long inlet. The low dissolved lignin concentration could result from its distance from river input. The greater distance of source to station, compared to other stations, may allow more time for dissolved lignin settle out of water column or be consumed by bacteria. The decreasing dissolved lignin concentrations are also seen in studies done on Russian Arctic coast and US continental margin (Benner et al. 2004; Moran et al. 1991). A dissimilar detail between the studies is the dissolved lignin concentrations near shore in Barkley Sound are 7.3 times lower compared to the averaged 26.5 μgL^{-1} value for the drainage basin of Russian rivers. One potential cause for the difference is that Russian rivers lignin sources consist of large areas of tundra, peat and boreal forests (Benner et al. 2004; Lobbes et al. 2000). The soil contents of tundra, peat and boreal forest soil hold large percentages of OC (Weintraub and Schimel 2003; Lobbes et al. 2000). The high OC content would lead to a larger pool of vegetation to allow the collection on large amounts of lignin in Russian rivers. The soils surrounding Barkley Sound are poorly developed which is further promoted by extensive logging.

These soil conditions may only provide a small vegetation pool to supply lignin to Barkley Sound.

Lignin found in marine samples are robust enough to indicate their terrestrial plant origins. This is shown by the clustering of each station's S/V to C/V ratios near the origin (Fig. 2). The close proximity of the cluster to the origin indicates that the dissolved lignin in Barkley Sound is mainly derived from evergreen wood (Fig. 2). But an influence of dissolved lignin from needles and angiosperm wood can also be seen. C from needles and S from angiosperm wood move the cluster away from the origin. The average C/V ratio 0.11 indicates that 27.5% of the dissolved lignin came from needles and 72.5% came from evergreen wood. The average S/V ratio 0.28 indicates that 72% of lignin from wood came from evergreen and 28% from angiosperm. A previous study on lignin in sediments within other Vancouver Island fjords also indicates a woody gymnosperm origin (Walsh et al. 2008). The lignin signatures in the sediments and water-column correlate well with Vancouver Island being dominated by Evergreen forests. The same signature occurs even though the sediment and water-column samples differ in geographical location. This may imply that all Vancouver Island estuarine water and sediment lignin signatures portray similar plant sources and can be verified with future Vancouver Island studies.

Dissolved lignin is known to have a higher degree of degradation than particulate lignin (Hernes et al. 2007). A large degree of lignin degradation can occur through microbes and white-rot fungus (Crawford and Crawford 1980; Hedges et al. 2003). Degradation occurs when lignin aldehydes (Al) are converted to acid derivatives (Ad) and is measured using Ad/Al ratios (Rezende et al. 2010). The degradation of vanillyl phenols (Ad/Al(v)) in Barkley Sound, with an average of 1.01, is similar to those seen in U.S. east coastal waters, Russian rivers and Sepetiba Bay in RJ, Brasil (Moran et al. 1991; Benner et al. 2004; Rezende et al. 2010). Biological and physical processes have a preference for syringyl to vanillyl phenols which causes syringyl phenols to have a higher Ad/Al. (Louchouarn et al. 1997). This can be seen in Barkley Sound by comparing the of 2.6 Ad/Al(s) to 1.01 Ad/Al(v) averages. An interesting phenomenon occurs with the two highest Ad/Al(s) of 6 being located near fish pens. A study states that there is increased bacterial activity around fish pens (Sakami et al. 2003). With increased bacterial activity, the lignin passing through fish pens likelier to degrade than lignin passing through nearby waters. Aquatic farms, not just fish farms, may pose as hot spots for increased lignin degradation. The extensive areas of aquatic farming include many estuaries and fjords in the Northwest and other parts of the world. For future studies in these areas may need to take into account the degradation of syringyl, vanillyl and cinnamyl phenols. Syringyl and cinnamyl phenol may need to be especially accounted for because past methods using them in lignin ratios may be affected.

Degradation of lignin decreases the individual lignin phenol concentrations which in turn affect the accuracy of S/V to C/V plots to illustrate the plant source of the lignin. As syringyl and cinnamyl phenols degrade faster than vanillyl phenols the S/V and C/V ratios decrease. This causes the points on the S/V to C/V graph to plot nearer to the origin (Fig. 2). The plotted points do not accurately represent the ratios that would be seen in the undegraded lignin. Without taking into account degradation the points may falsely portray lignin ratios that are more representative of evergreen wood. In contrast of lignin phenol ratios would be less representative of needle, angiosperm wood, leaf or grass.

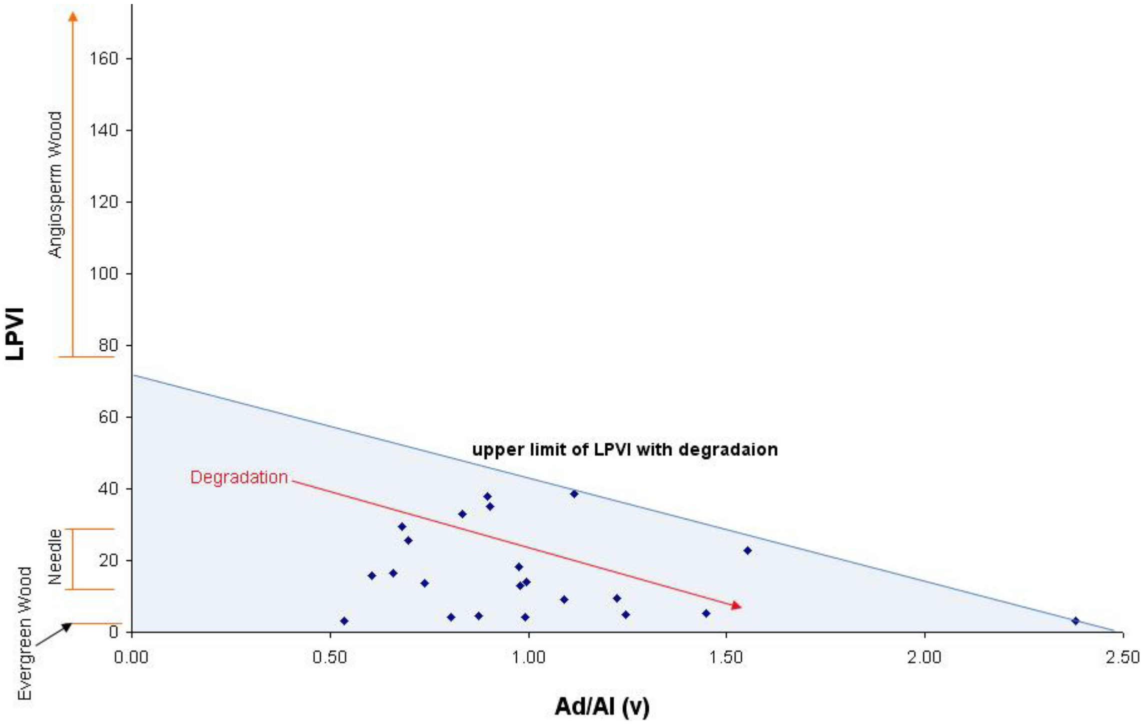


Figure 2
The effect of degradation on of vanillyl lignin and the upper estimate LPVI values.

A plot of the Lignin Phenol Vegetation Index (LPVI) to Ad/Al ratio is a way to look at the effects of lignin degradation on unaltered lignin signature (Rezende et al. 2010)(Fig. 3). The LPVI gives specific values to plant tissues based on their syringyl, vanillyl and cinnamyl lignin phenols (Tareq et al. 2004). Figure 3 shows that as the Ad/Al(v) ratio increased there was a decrease in LPVI. There is an upper limit were no Ad/Al(v) ratio exists above the LPVI. When the upper limit is traced back to undegraded vanillyl phenols (Ad/Al = 0) an LPVI of 75 is acquired. This LPVI indicates the highest possible value with no influence of lignin degradation. Taking an irradiative approach, the new LPVI value is used to identify upper limits for S/V and C/V, 0.41 and 0.31 respectively (Fig. 2). The limits create a box around the points that demonstrate the most conservative estimates at which the S/V and C/V ratios can be before the affect of lignin degradation.

These estimates bound 78% of the lignin phenols to originate from needle tissue instead of wood and 50% to originate from angiosperm wood instead of evergreen wood. The new approach to account for degradation be further applied too lignin studies involving particulate matter, water-column and sediment.

Conclusions

- The source of lignin for Barkley Sound is smaller compared to other coastal setting, but has a higher degree of degradation.

- Traditional methods of S/V to C/V lignin phenol plot characterize terrestrial OC in Barkley Sound to originate mainly from evergreen wood.

-Comparing the LPVI to Ad/Al ratios helps understand determine the extent of degradation on signals from lignin phenols. Such that a generous signature of lignin phenols at most 4/5 the lignin could come from needle instead of wood, while only 1/2 could originate from deciduous wood instead of evergreen wood.

-Degradation alters the source terrestrial plant signal given by dissolved lignin phenols. Therefore, studies using traditional S/V to C/V lignin phenol plot need to consider lignin degradation.

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